Canopy and understory nitrogen additions differently affect soil microbial residual carbon in a temperate forest

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Abstract

Atmospheric nitrogen (N) deposition in forests can affect soil microbial growth and turnover directly through increasing N availability and indirectly through altering plant-derived carbon (C) availability for microbes. This impacts microbial residues (i.e., necromass), a major component of soil organic C. Previous studies in forest ecosystems have so far focused on the impact of understory N addition on microbes and microbial residues, but the effect of N deposition through plant canopy, the major pathway of N deposition in nature, has not been explicitly explored. We investigated whether and how the quantity and modes (canopy vs. understory) of N addition affect soil microbial residues in a temperate broadleaf forest under 10-yr N additions. Our results showed that N addition enhanced soil amino sugars and microbial residual C concentrations, especially in the topsoil under high N addition. Canopy N addition had stronger positive effects on soil amino sugars and microbial residual C than understory N addition in the subsoil, implying that the indirect pathway via plants plays a more important role. Also, neither canopy nor understory N addition significantly affected soil microbial biomass and microbial community structure, suggesting that enhanced microbial residues under N deposition stemmed from increased microbial biomass turnover. These findings indicate that understory N addition underestimates the impact of N deposition on soil microbial residues, suggesting that canopy related processes should also be considered in temperate forest ecosystems.

Keywords: microbial residues, amino sugars, nitrogen deposition, canopy interception, soil microbial community, microbial biomass turnover, nitrogen addition modes

1. INTRODUCTION

The soil organic carbon (SOC) pool is twice larger than the amount of current atmospheric C, and a small change in SOC may significantly affect the atmospheric CO_2 concentration (Smith, 2012). It is assumed that high atmospheric CO_2 may increase plant growth and then soil C storage, especially if it is accompanied with an increasing reactive N input (Ackerman et al., 2019; Stevens, 2019). Human activities, N fertilization in particular, have increased global reactive N input and N deposition (Zaehle et al., 2011). Elevated N deposition alters soil N availability for plants and microbes (Stevens et al., 2018). Because N is the primary limiting factor of plant growth in terrestrial ecosystems, N promotion of plant growth likely enhances microbial growth and allows microorganisms to transform more plant-derived C into microbial residues (Ataka et al., 2020; Wang et al., 2022). Also, N input can modify the composition of the soil microbial community, altering soil organic matter decomposition (Morrison et al., 2016). Altered microbial growth and biomass turnover would affect microbial residues such as amino sugars (Freppaz et al., 2014; Miltner et al., 2011; Ni et al., 2020). Microbial residual C is an important component of SOC, accounting for up to half of the stable SOC pool in terrestrial ecosystems (Liang et al., 2019; Wang et al. 2021a). Therefore, the knowledge of N deposition effects on soil microbial residues is vital to comprehensively understand the soil C dynamics under N deposition (Gilliam et al., 2019).

Many studies have examined the responses of soil microbial residues to atmospheric N deposition (Averill et al., 2018; Griepentrog et al., 2014). Amino sugars, as specific biomarkers of microbial residues in soils (Joergensen et al., 2018), have been extensively used to assess the contribution of microbial-derived C to SOC and their responses to climate change (Liang

et al., 2019; Malik et al., 2020). Generally, changes in soil microbial biomass, microbial community structure, and biomass turnover all can alter microbial residues (Wang et al., 2021b). In forest ecosystems, N deposition has been shown to enhance soil microbial residual C in some studies (Liao et al., 2022; Zhang et al., 2023), but not in others (Ma et al., 2021; Yuan et al., 2021; Zhang et al., 2016). Also, N addition can reduce fungal residues in soil as high mineral N often suppresses fungi (Ma et al., 2020; Treseder et al., 2008). These inconsistent results may stem from the contrasting responses of soil microbial groups (bacteria and fungi) to N addition. Alternatively, they may be due to various responses of microbial turnover and soil organic matter decomposition to changes in soil physicochemical properties (e.g., soil pH) and plant associated processes (e.g., quantity and/or quality of rhizodeposition and/or litter) caused by N addition (Khan et al., 2016; Wang et al., 2023). Also, the rate of N additions impact soil microbial biomass and community structure (Frey et al., 2004; Tian et al., 2022). For instance, low N addition usually increases microbial biomass in forests (Waldrop et al., 2004), but high N addition may reduce microbial biomass and suppress microbial activity and microbial decomposition of organic matter (Jing et al., 2021; Meunier et al., 2016). However, the exact mechanisms underpinning N rate effects on microbial residues are still unclear in temperate forests.

The current understanding of atmospheric N deposition effects on forest SOC and microbial residues is mainly built on the studies of understory N additions (Chang et al., 2019; Gurmesa et al., 2022). Understory N addition affects soil microbial biomass and community structure mainly via direct N effects on microbes and soil physicochemical properties (Chen et al., 2023; Jia et al., 2020). Understory N addition may overestimate the N effect on soil

89 microbes because of the absence of canopy N interception (Zhang et al., 2015). Forest canopy
90 may retain a remarkable proportion of incoming atmospheric N and alter the quality and
91 quantity of N that enters into soils (Guerrieri et al., 2021). For example, canopy leaves, twigs
92 and branches could absorb the N from canopy addition (Houle et al., 2015). These canopy-
93 associated processes will indirectly affect soil microbial biomass, microbial community
94 structure, and soil organic matter decomposition through altering litter properties, soil-derived
95 N absorption and C availability to microbes. It was reported that canopy N interception
96 mitigated the direct impact of N on soil biota (Liu et al., 2020). Moreover, N addition modes
97 also affect the magnitude of N-induced changes in soil physicochemical properties and plant
98 chemical properties. Therefore, the mode of N addition may exert a critical control over the N
99 effects on soil ecological and plant physiological processes that affect C cycling in forest
100 ecosystems (Lu et al., 2021; Yu et al., 2019).

101 To explore the effects of N deposition modes and quantities on forest ecosystem structure
102 and functions, we initialized a long-term field manipulation study with two modes (canopy and
103 understory additions) and three N-addition rates in a temperate forest in 2013. As a component
104 of this extensive field study, we examined the effects of N deposition on soil microbes and
105 microbial C residues in soil. Because forest litters have high C:N ratios and microbes in forest
106 soils are also N-limited, we predict that N addition alleviates N limitation on microbes and
107 increases microbial biomass and microbial residues in soil (Hypothesis 1). Given that canopy
108 N interception reduces the quantity of N that directly enters soil, we also expect that understory
109 N addition has stronger effects on microbial residues than canopy N addition (Hypothesis 2).

110 **2. MATERIALS AND METHODS**

2.1. Site description

This study was conducted at the Dabieshan National Field Observation and Research Station of Forest Ecosystem (31°46'-31°52'N, 114°01'-114°06'E), located in the Jigongshan National Nature Reserve, in Henan Province of China. The climate is characterized by a subtropical-warm temperate climate. In this studied region, the mean annual precipitation was approximately 1119 mm and the mean annual temperature was 15.2 ℃ (Zhang et al., 2015). The temperate deciduous broadleaf forest is a typical vegetation type, and the dominant tree species include *Liquidambar formosana* Hance, *Quercus acutissima* Carruth, and *Quercus variabilis* Bl. The forest soil is classified as yellow-brown sandy-loam soil (Liu et al., 2020). The background wet N deposition rate was approximately 20 kg N ha^{-1} yr^{-1} at this study site (Zhang et al., 2015).

2.2. Experimental design

This experiment included understory N addition (UN) and canopy N addition (CN), and was set as a randomized block design with four blocks. Each block contained five treatments: CT (control, no N addition), CN25 (canopy N addition at 25 kg N ha^{-1} yr^{-1}, low-N), CN50 (canopy N addition at 50 kg N ha^{-1} yr^{-1}, high-N), UN25 (understory N addition at 25 kg N ha^{-1} yr^{-1}, low-N), and UN50 (understory N addition at 50 kg N ha^{-1} yr^{-1}, high-N). There were 20 plots in total, and one plot was a circle with a diameter of 34 m, which was surrounded by a > 20 m buffer zone. Cement boards were inserted into the soil to a depth of 50 cm to separate each plot from adjacent plots. The N form in the treatments was NH_4NO_3 solution, which was sprayed monthly from April to October (seven times per year). All treatments were initiated in April 2013. More details on this experimental design were included in Zhang et al. (2015).

2.3. Soil sampling and physicochemical properties

Soil samples were collected from the depths of 0-10 cm and 10-20 cm in July 2022, respectively, corresponding to the 10th year of N addition. In each plot, five cores with the same depth were sampled by an auger (3.0 cm in diameter) from five randomly selected microsites and bulked into one pooled sample. There were 40 soil samples in total. Visible plant materials and roots were removed by hand. All fresh soil samples were sieved using a 2 mm soil sieve and taken back to the laboratory. Each soil sample was divided into two subsamples: one was air-dried for analysis of soil physicochemical properties, and the other was stored at -20℃ for determination of microbial biomass and community structure.

Soil pH was measured in a slurry (soil: water = 1: 2.5, w/v) with a pH meter (FiveEasy Plus^{TM} FE28, Mettler Toledo). SOC, soil total nitrogen (TN) and total phosphorus (TP) concentrations were determined by the concentrated sulfuric acid-potassium dichromate external heating method, the concentrated sulfuric acid digestion-phenol blue colorimetric method, and the concentrated sulfuric acid digestion-molybdenum antimony anti-colorimetric method, respectively (Lu, 1999).

2.4. Soil microbial biomass and community structure

Soil microbial community was characterized by the phospholipid fatty acids (PLFAs) method (Bossio & Scow 1998). Concentration of each PLFA was calculated based on 19:0 internal standard concentration. The PLFAs i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, a18:0, i18:0, a19:0, 16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω7, cy17:0, and cy19:0 were used to indicate bacterial biomarkers. The PLFAs 18:1ω9c, 18:2ω6,9c, and 18:3ω6,9,12c were applied to denote fungal biomarkers. The PLFA 16:1ω5c was considered as arbuscular mycorrhizal fungal (AMF)

biomarker. The PLFAs 10Me 16:0, 10Me 17:0, and 10Me 18:0 were used as actinomycetes biomarkers. Total microbial biomass was represented by the sum of bacterial, fungal, AMF, and actinomycetes biomarkers. Soil microbial community structure was represented by fungal: bacterial biomass ratio (F:B ratio) (Frostegård & Bååth 1996).

2.5. Soil amino sugars and microbial residual C

Soil amino sugar (ASs) concentrations, including murmic acid (MurN), galactosamine (GalN), and glucosamine (GluN), were determined as described by Indorf et al. (2011). In brief, amino sugars were hydrolyzed, extracted, and derivatized with ortho-phthaldialdehyde, determined by high-performance liquid chromatography (Dionex Ultimate 3000, Thermo Fisher Scientific). The detailed relevant information was described by Yuan et al. (2021). Microbial residual C (MRC) was calculated by the following formulas:

$$\text{F-GluN } (\mu g\ g^{-1}) = \text{total GluN } (\mu g\ g^{-1}) - 2 \times \text{MurN } (\mu g\ g^{-1}) \times (179.2/251.2) \quad (1)$$

$$\text{Fungal MRC } (\mu g\ g^{-1}) = \text{F-GluN} \times 9 \quad (2)$$

$$\text{Bacterial MRC } (\mu g\ g^{-1}) = \text{MurN} \times 45 \quad (3)$$

$$\text{Total MRC } (\mu g\ g^{-1}) = \text{Fungal MRC} + \text{Bacterial MRC} \quad (4)$$

Where F-GluN is fungi-derived GluN. Fungal MRC, bacterial MRC, and total MRC are fungi-derived, bacteria-derived, and total microbial residual C, respectively. It was assumed that MurN and GluN occurred at a 1 to 2 molar ratio in bacterial cell walls (Engelking et al., 2007). Where 179.2 and 251.2 are the molecular weights of GluN and MurN, respectively (Shao et al., 2017). Where 9 and 45 are conversion factors (Appuhn & Joergensen, 2006).

2.6. Data analysis

One-way ANOVA was employed to examine the effects of N deposition on concentrations of soil total amino sugar (total ASs), MurN, GalN, GluN, fungal MRC, bacterial MRC, total MRC, and the ratio of fungal to bacterial MRC. The effects of N deposition on the contributions of total ASs, fungal MRC, bacterial MRC, and total MRC to SOC were tested by one-way ANOVA as well. The N deposition impacts on soil physicochemical properties (pH, SOC, TN, TP) and soil microbial parameters (the biomasses of fungi, bacteria, AMF, actinomycetes, and total microbes, and the F:B ratio) were examined by one-way ANOVA. Multiple comparison analyses (LSD) were used after one-way ANOVA. Pearson correlation analysis was performed to assess the relationships of measured soil microbial residues (amino sugars and residual C) with soil physicochemical properties and soil microbial parameters (microbial biomass and community structure). All statistical analyses were carried out with SPSS 18.0 (SPSS, Chicago, Illinois, USA), and results were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1. Soil amino sugars and microbial residual C

High-N addition (50 kg N ha^{-1} yr^{-1}) significantly enhanced soil amino sugar concentrations in the 0-10 cm layer, regardless of canopy or understory N addition (Figure 1a,c). Higher concentrations of MurN, GluN, and total amino sugars were observed in CN50 and UN50, compared with that in CT. They increased by 43.50-53.36% and 58.09-62.58% for CN50 and UN50, respectively. The concentration of GalN was significantly higher in UN50 not CN50 than that in CT ($p = 0.010$ and 0.066, respectively). Low-N addition (25 kg N ha^{-1} yr^{-1}) did not significantly increase soil amino sugar concentrations including MurN, GalN, GluN, and total

amino sugars, no matter canopy or understory N addition. In the 10-20 cm layer, canopy N addition increased soil amino sugar concentrations, especially canopy high-N addition (Figure 1b,d). Specifically, the concentrations of MurN, GluN, and total amino sugars in CN50 were significantly higher than that in CT. Also, the MurN concentration in CN25 was higher than that in CT ($p = 0.030$). Neither understory high-N nor low-N addition significantly affected soil amino sugar concentrations in the 10-20 cm layer (Figure 1b,d), and the GalN concentration was not significantly affected by canopy or understory N addition. The mean contributions of total amino sugars to SOC were 3.92-4.63% and 4.37-5.20% in the 0-10 cm and 10-20 cm layers, respectively, these were not altered by canopy or understory N addition (Figure 2). Overall, the contribution of total amino sugars to SOC was greater in the 10-20 cm than that in the 0-10 cm layer ($p = 0.023$, Figure 2).

Both the canopy and understory high-N additions significantly increased soil microbial residual C (MRC) concentration in the 0-10 cm layer. Specifically, the fungal MRC, bacterial MRC, and total MRC concentrations were significantly higher in the CN50 and UN50 than that in the CT (Figure 3a). Canopy and understory low-N additions did not significantly affect the microbial residual C as fungal MRC, bacterial MRC, and total MRC. Besides, the ratio of fungal to bacterial MRC was not significantly influenced by canopy and understory N addition (Figure 3a). In the 10-20 cm layer, canopy not understory N addition increased soil MRC concentrations, especially canopy high-N addition (Figure 3b). The concentrations of fungal, bacterial, and total MRC were significantly higher in CN50 than that in CT. The bacterial MRC concentration in CN25 were also significantly higher than that in CT. While no matter low- or high-N, understory N addition did not significantly affect microbial residue C (Figure 3b). The

ratios of fungal to bacterial MRC were not also affected by canopy and understory N additions (Figure 3b). The contributions of MRC (fungal, bacterial, and total MRC) to SOC were not significantly altered by canopy and understory N additions in the 0-10 cm and 10-20 cm layers as well (Figure 3c,d).

3.2. Soil microbial biomass and community structure

In the 0-10 cm layer, N addition did not significantly affect soil microbial biomass such as the biomasses of fungi, bacteria, actinomycetes, AMF, and total microbes (Table 1; all $p > 0.05$). The F:B ratio was not significantly affected by N addition, regardless of canopy or understory N addition. In the 10-20 cm layer, there were no significant differences in soil microbial biomass including the biomasses of bacteria, fungi, actinomycetes, AMF, and total microbes, and the F:B ratio (Table 1; all $p > 0.05$). The biomasses of bacteria, fungi, actinomycetes, AMF, and total microbes were also not significantly higher under the N addition treatments relative to CT in the 0-10 and 10-20 cm layers.

3.3. Soil physiochemical properties

In the 0-10 cm layer, N addition altered soil pH and soil total N concentration. The soil pH was lower in CN25, UN25, and UN50 than that in CT (Table 2). Higher soil N concentration was observed in CN25, CN50, and UN50 than in CT. The SOC concentrations were slightly higher in the treatments of N addition than in CT, although the differences were not statistically significant ($p = 0.472$). There was no significant difference in soil total P concentration among different treatments ($p = 0.934$, Table 2). In the 10-20 cm layer, the patterns of soil pH, SOC, and total P in all treatments were the similar to that in the 0-10 cm layer. Specifically, soil pH

was significantly lower in CN25, UN25, and UN50 than that in CT. No significant differences in SOC and total P concentrations were found among different treatments. The treatments did not differ in soil total N concentrations, although they were slightly higher in N addition treatments than that in CT in the 10-20 cm layer (Table 2).

3.4. Linkages between microbial residues and soil properties

Soil physicochemical properties and microbial biomass affected soil amino sugars. The concentrations of amino sugars including MurN, GalN, and GluN, and total ASs were negatively correlated to soil pH, but positively correlated to SOC, TN, and biomasses of bacteria, fungi, actinomycetes, and total microbes in the 0-10 cm layer (Figure 4a). In the 10-20 cm layer, the concentrations of MurN, GalN, GluN, and total ASs were not affected by soil pH, but positively associated with SOC, TN (except GalN), and biomasses of bacteria, AMF, actinomycetes and total microbes (Figure 4b). In addition, the contributions of amino sugars (MurN, GalN, GluN, and total ASs) to SOC were only negatively correlated to SOC in the 0-10 cm and 10-20 cm layers. The AMF biomass negatively affected the contributions of total amino sugars and GalN to SOC in the 0-10 cm layer (Figure 4a). However, other soil parameters (i.e., pH, TN, TP, and biomasses of bacteria, fungi, AMF, actinomycete, and total microbes) did not markedly alter the contributions of amino sugars to SOC in the two studied soil layers (Figure 4a,b).

The microbial residual C was significantly affected by soil properties in the two studied soil layers. Fungal MRC, bacterial MRC, and total MRC were negatively correlated to soil pH, but positively correlated to SOC, TN, and biomasses of bacteria, fungi, actinomycetes, and total microbes in the 0-10 cm layer (Figure 4a). In the 10-20 cm layer, there was no significant

correlations between microbial residual C (fungal MRC, bacterial MRC, and total MRC) and soil pH. While fungal MRC, bacterial MRC, and total MRC were positively associated with SOC, TN, and biomasses of bacteria, AMF, actinomycetes, and total microbes (Figure 4b). Furthermore, the contributions of microbial residual C to SOC were only negatively affected by SOC in the 0-10 cm and 10-20 cm layers (Figure 4a,b). The ratio of fungal to bacterial MRC was not affected by the studied soil physiochemical properties and microbial biomass in the 0-10 cm and 10-20 cm layers (Figure 4a,b).

4. DISCUSSION

4.1. Dominant determinants of response of soil microbial residues to N addition

The changes in soil microbial biomass following N addition can reveal the rapid response of microbial growth and metabolism to N addition (Ma et al., 2020). Nitrogen addition had a minor effect on soil microbial biomass in this study, which did not support our first hypothesis that soil microbial biomass would increase under N addition. Additionally, the F:B ratio was not altered by N addition. The lack of response suggests that N was not the most important limiting factor. Microbial community variations could be limited by soil moisture or availability of other nutrients, or other environmental factors (Liu et al., 2022). The effect of N on soil microbial biomass was thus not significant in this study but further studies and long-time monitoring of soil microbial dynamics are needed.

In this study, high N not low N deposition significantly enhanced individual and total amino sugar concentrations in the topsoil, which partly supported our hypothesis that N addition would increase microbial residues. The response of microbial residue accumulation to

N deposition is related to N addition rate (Tian et al., 2022). Low N addition did not cause changes in soil physiochemical properties and soil microbial biomass, possibly due to the soil capacity to buffer disturbance to some extent (Lo Cascio et al., 2021). Additionally, the significantly positive correlations between soil amino sugars and microbial biomass were detected in this study, although high-N addition did not significantly affect soil microbial biomass. The soil physiochemical properties such as soil pH, SOC, and soil TN displayed significant effects on amino sugars, which could change soil amino sugars via altering soil microbial turnover (Fig. 5) (Brabcová et al., 2018). Since amino sugars provides the information on time-integrated microbial community (Glaser et al., 2004), we propose that the changes in amino sugar concentration could be substantially resulted from the alteration of soil microbial turnover rather than microbial biomass.

Soil microbial residual C is regulated by soil microbial residues deposition and decomposition (Fernandez et al., 2019; Freedman et al., 2016; Shao et al., 2021). Soil microbial biomass is a key factor determining soil microbial residues deposition. In this study, although N addition (canopy and understory N addition) did not markedly alter soil microbial biomass and community structure, the positive correlations between microbial residue C and microbial biomass were observed. Microbial residual C is a long-term accumulated product from soil microbes and is not fully equal to living microbial biomass (Camenzind et al., 2023; Zhang et al., 2021). In the long term, a non-significant difference in microbial biomass also may generate an obvious discrepancy in microbial residual C. The previous study reported that N addition did not increase the activity of residue-decomposing enzymes (Yuan et al., 2021). So it is more probably ascribed to the increase in microbial residue deposition rather than reduction in

305 microbial residue decomposition. High N addition increases soil N availability and plant
306 productivity (Lebauer & Tresder, 2008; Li et al., 2021). The enhanced plant productivity needs
307 more nutrient supply (Giardina et al., 2003). To obtain more nutrients, fast turnover rate of
308 microbial biomass thus would be triggered and result in more microbial residue C accumulation.

309 **4.2. Differentiated responses of soil microbial residues to N addition modes**

310 Canopy and understory N additions exhibited contrast effects on soil microbial residues in this
311 study. Amino sugar and microbial residual C concentrations were enhanced by canopy high-N
312 addition in two soil layers, but increased by understory high-N addition only in the 0-10 cm
313 layer. The result was not consistent with our hypothesis that the effect of understory N addition
314 on microbial residues is greater than that of canopy N addition. It could be attributable to the
315 different impact pathways of canopy and understory N additions on soil microbial residues (Fig.
316 5).

317 The indirect pathway via plants could dominate the N effect on soil microbial residues
318 (Figure 5). Canopy N addition directly influences plants and then indirectly influences soil
319 microbes through altering the traits of leaf, litter, and root of canopy trees, and C availability
320 to soil microbes (Cantarel et al., 2015; Feng et al., 2022; terHorst & Zee, 2016). For instance,
321 the increase in leaf N concentration induced by canopy N addition may improve leaf
322 photosynthesis (Li et al., 2021; Wang et al., 2021c). Whereafter, the amount of photosynthates
323 allocated to belowground plant parts would be enhanced (Farrar & Jones, 2000; Hendricks et
324 al., 2000). The fine root biomass and production were significantly higher with canopy N
325 addition than with understory N addition in the same experimental plots as used in this study
326 (Li et al., 2021). The boosted fine root biomass and production may stimulate soil microbial

activities and promote microbial turnover to obtain more nutrients. By contrast, the N from understory addition is sprayed onto the forest floor and go directly into the soil (Figure 5). Understory low-N addition did not cause the significant changes in soil properties and trigger the response of soil microbes. The low N with understory addition may not be enough to lead to a significant and timely response of canopy trees (Forsmark et al., 2021). Alternatively, the negative effect of acidification could be offsetting the positive effect of enhanced resources.

5. CONCLUSIONS

Canopy and understory N additions differed in effects on soil microbial residues. Both canopy and understory high-N additions enhanced soil amino sugar and microbial residual C concentrations in the topsoil. Whereas, in the subsoil, canopy but not understory N addition exhibited a positive effect on soil amino sugars and microbial residue C, especially canopy high-N addition. Moreover, neither canopy nor understory N addition significantly affected soil microbial biomass and community structure. This experimental evidence suggests that microbial turnover may make a notable contribution to microbial residue accumulation relative to microbial biomass, and the N addition impact on soil microbial residues relies on simulation modes of N deposition and N addition rates. These findings indicate understory N addition underestimates the impact of N deposition on soil microbial residues, and N addition effects on plant canopy, plant growth, and microbial turnover exert a major control over the formation of microbial-derived SOC. We thus propose canopy related processes should be considered when assessing and predicting the effect of N deposition on C sink function in temperate forest ecosystems.

AUTHOR CONTRIBUTIONS

Yuanqi Chen, Shirong Liu and Shenglei Fu conceived the study; Yuanqi Chen, Yu Zhang, Xu Zhang, and Xiaowei Li conducted this study; Yuanqi Chen, Teng Feng, and Quan Chen analyzed the data; Yuanqi Chen, Yu Zhang and Xu Zhang wrote the manuscript; Yuanqi Chen, Shirong Liu, Carly Stevens and Shuijin Hu revised the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available in the main text or the Supporting Information and raw data are available upon request from the corresponding author.

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Figure legends

Figure 1 Soil amino sugar concentrations in all treatments in the 0-10 cm (1a, 1b) and 10-20 cm (1c, 1d) layers. GalN, GluN, MurN, and Total ASs refer to galactosamine, glucosamine, muramic acid, and total amino sugars. CT, CN25, CN50, UN25, and UN50 stand for the treatments of control, canopy N addition at 25 kg N ha^{-1} yr^{-1} and 50 kg N ha^{-1} yr^{-1}, understory N addition at 25 kg N ha^{-1} yr^{-1} and 50 kg N ha^{-1} yr^{-1}, respectively. Values are means ± SE, n = 4. Different lowercase letters indicate significant differences in the same amino sugar among different treatments in the same soil layer at the $p = 0.05$ level.

Figure 2 The contribution of total amino sugars (ASs) to SOC in all treatments in the 0-10 cm and 10-20 cm layers. Values are means ± SE, n = 4. Different capital letters on the line indicate significant differences between 0-10 cm and 10-20 cm soil layers at the $p = 0.05$ level. See Figure 1 for abbreviations.

Figure 3 Microbial residual C (MRC) and their contribution to SOC in all treatments in the 0-10 cm (3a, 3c) and 10-20 cm (3b, 3d) soil layers. B-MRC, F-MRC, F/B-MRC, and T-MRC stand for the bacteria-derived MRC, fungi-derived MRC, the ratio of F-MRC to B-MRC, and total MRC, respectively. B-MRC/SOC, F-MRC/SOC, and T-MRC/SOC represent the contributions of B-MRC, F-MRC, and T-MRC to SOC, respectively. Values are means ± SE, n = 4. Different lowercase letters indicate significant differences in soil MRC among different treatments in the same soil layer at the $p = 0.05$ level. See Figure 1 for abbreviations.

Figure 4 The correlations between microbial residues (amino sugars and microbial residual C) and soil physiochemical properties in 0-10 cm (a) and 10-20 cm (b) soil layers. SOC, TN, TP, B, F, AMF, Act, and T-PLFAs stand for soil organic C, soil total N, soil total P, the biomasses of bacteria, fungi, arbuscular mycorrhizal fungi, actinomyces, and total microbes, respectively. See Figure 1 and Figure 3 for abbreviations.

Figure 5 Schematic diagram summarizing the effects of canopy N addition and understory N addition on soil microbial residues. The thin and thick arrows indicate the effects from one factor and many factors, respectively.

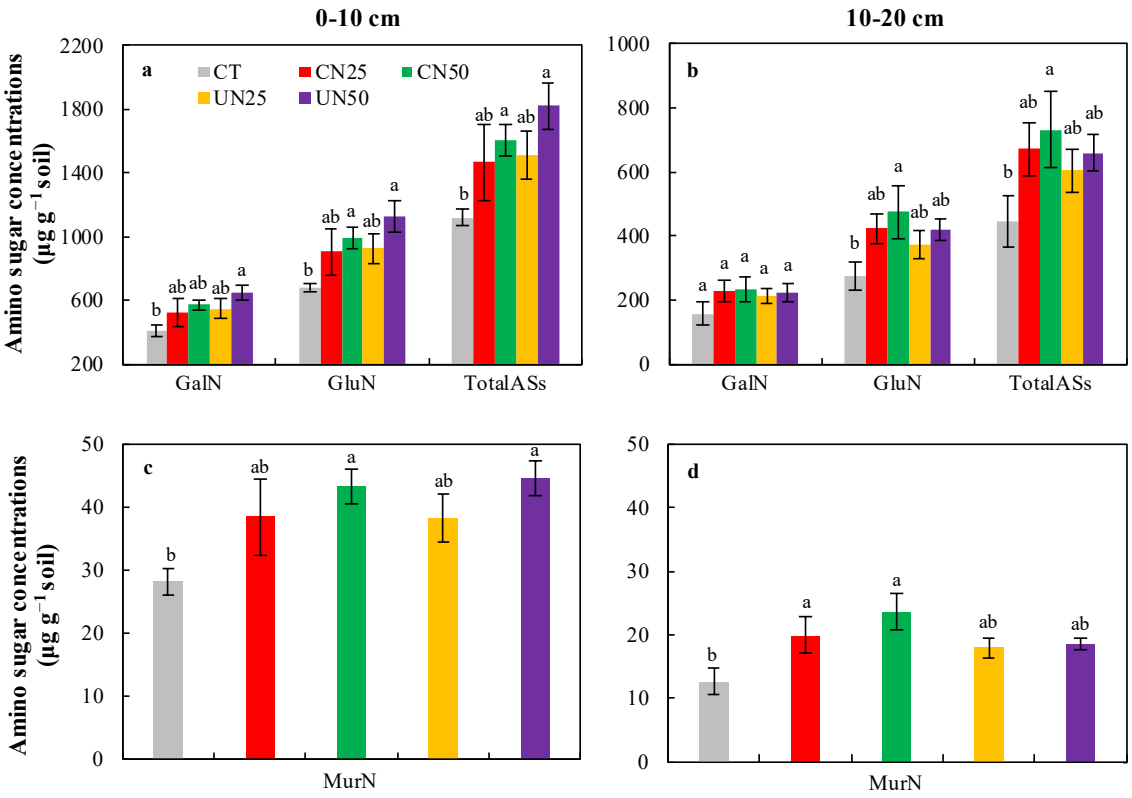
0-10 cm
10-20 cm
a
b
c
d
CT
CN25
CN50
UN25
UN50
Amino sugar concentrations (μg g⁻¹ soil)
GalN
GluN
TotalASs
MurN

Fig. 1

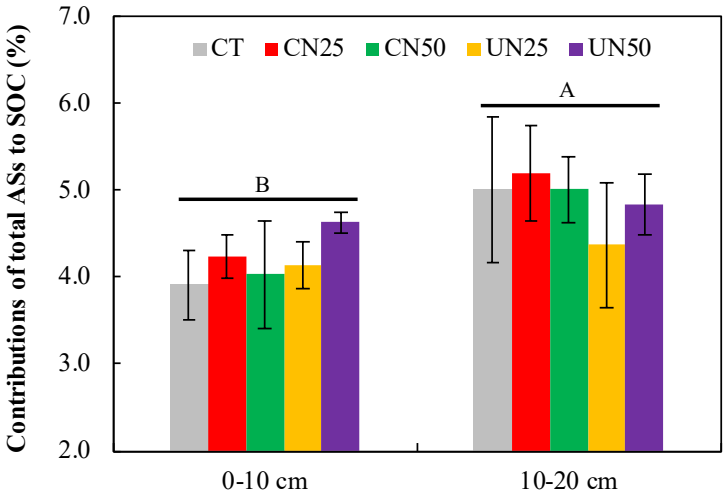
Contributions of total ASs to SOC (%)
7.0
6.0
5.0
4.0
3.0
2.0
CT
CN25
CN50
UN25
UN50
A
B
0-10 cm
10-20 cm

Fig. 2

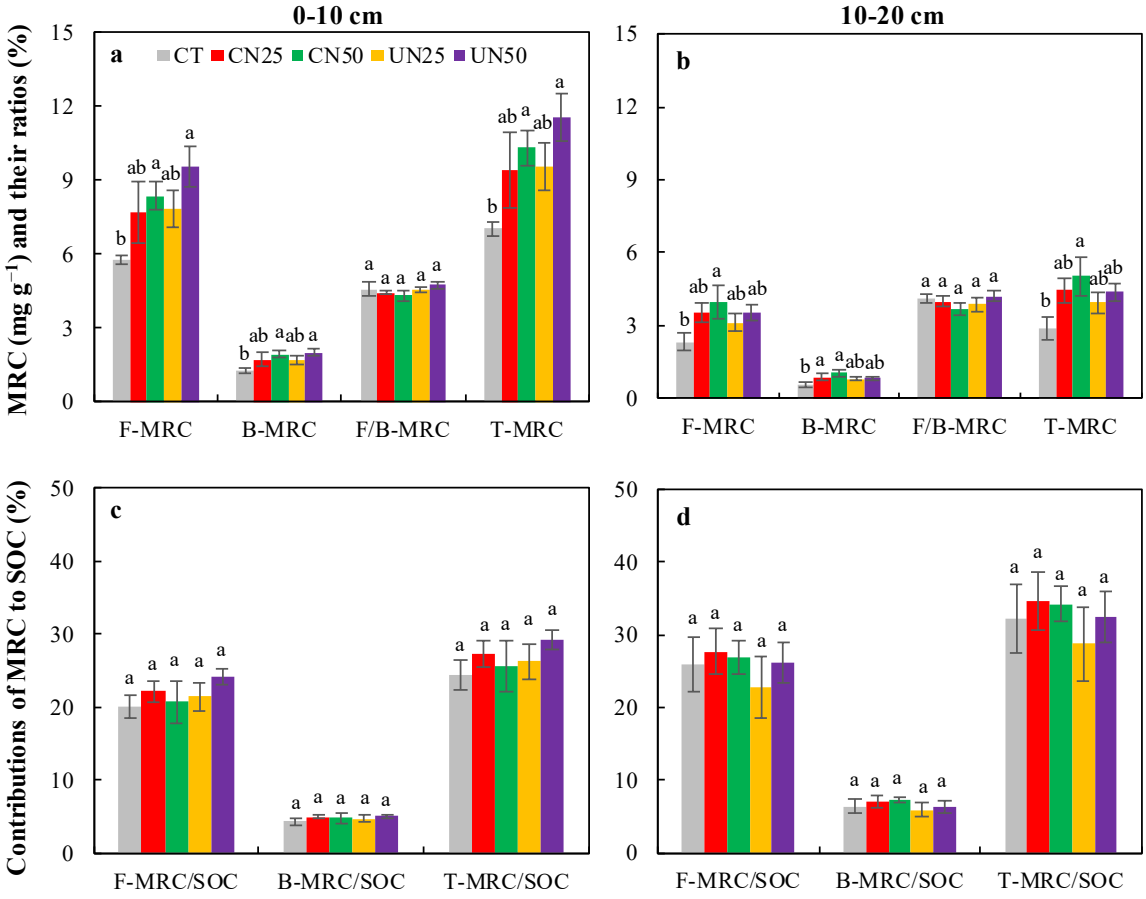
0-10 cm
10-20 cm
a
b
c
d
CT
CN25
CN50
UN25
UN50
MRC (mg g−1) and their ratios (%)
Contributions of MRC to SOC (%)
F-MRC
B-MRC
F/B-MRC
T-MRC
F-MRC/SOC
B-MRC/SOC
T-MRC/SOC

Fig. 3

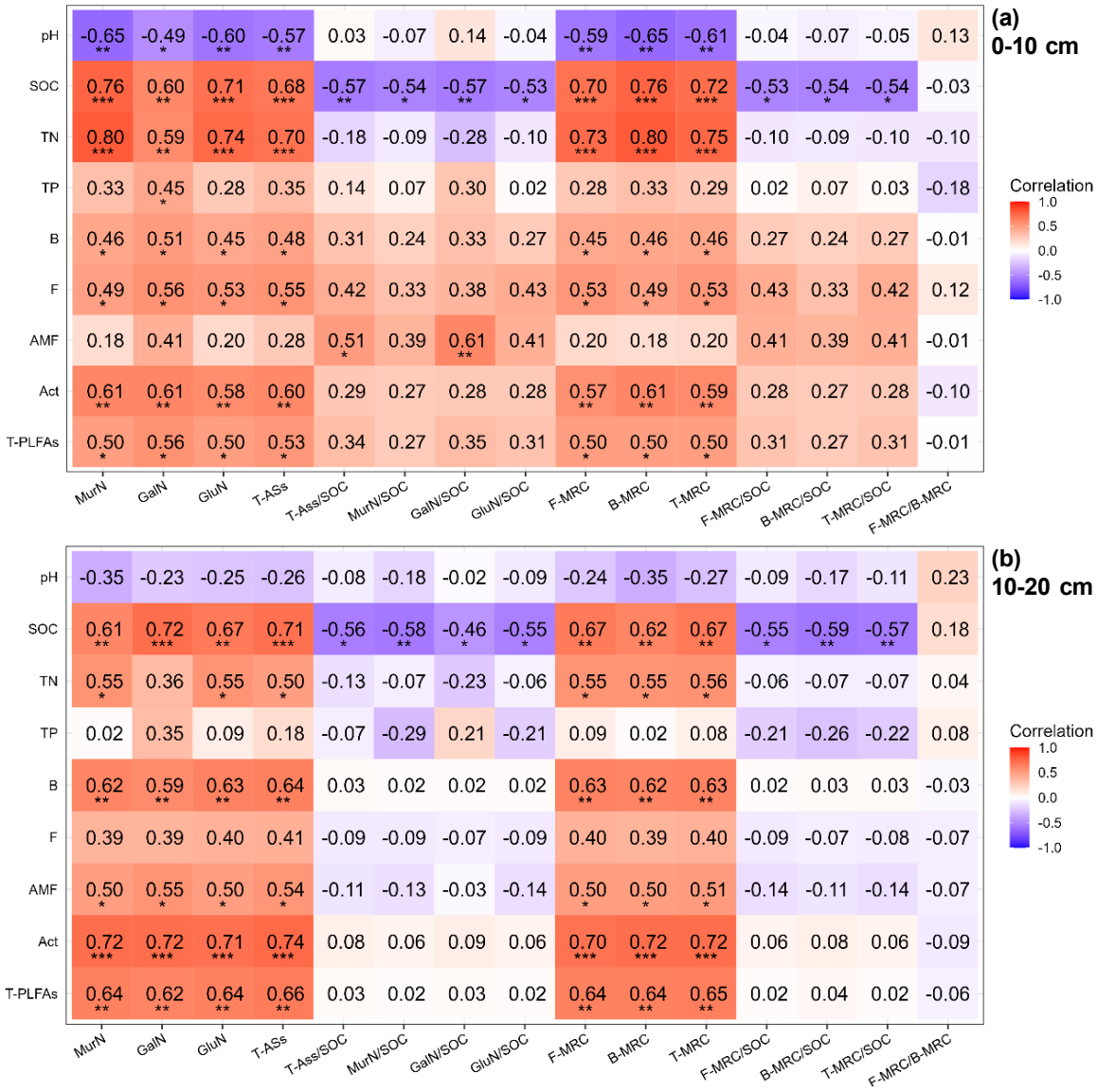
(a)
0-10 cm
pH -0.65** -0.49* -0.60** -0.57** 0.03 -0.07 0.14 -0.04 -0.59** -0.65** -0.61** -0.04 -0.07 -0.05 0.13
SOC 0.76*** 0.60** 0.71*** 0.68*** -0.57** -0.54* -0.57** -0.53* 0.70*** 0.76*** 0.72*** -0.53* -0.54* -0.54* -0.03
TN 0.80*** 0.59** 0.74*** 0.70*** -0.18 -0.09 -0.28 -0.10 0.73*** 0.80*** 0.75*** -0.10 -0.09 -0.10 -0.10
TP 0.33 0.45* 0.28 0.35 0.14 0.07 0.30 0.02 0.28 0.33 0.29 0.02 0.07 0.03 -0.18
B 0.46* 0.51* 0.45* 0.48* 0.31 0.24 0.33 0.27 0.45* 0.46* 0.46* 0.27 0.24 0.27 -0.01
F 0.49* 0.56* 0.53* 0.55* 0.42 0.33 0.38 0.43 0.53* 0.49* 0.53* 0.43 0.33 0.42 0.12
AMF 0.18 0.41 0.20 0.28 0.51* 0.39 0.61** 0.41 0.20 0.18 0.20 0.41 0.39 0.41 -0.01
Act 0.61** 0.61** 0.58** 0.60** 0.29 0.27 0.28 0.28 0.57** 0.61** 0.59** 0.28 0.27 0.28 -0.10
T-PLFAs 0.50* 0.56* 0.50* 0.53* 0.34 0.27 0.35 0.31 0.50* 0.50* 0.50* 0.31 0.27 0.31 -0.01
MurN GalN GluN T-ASs T-Ass/SOC MurN/SOC GalN/SOC GluN/SOC F-MRC B-MRC T-MRC F-MRC/SOC B-MRC/SOC T-MRC/SOC F-MRC/B-MRC
Correlation
1.0
0.5
0.0
-0.5
-1.0
(b)
10-20 cm
pH -0.35 -0.23 -0.25 -0.26 -0.08 -0.18 -0.02 -0.09 -0.24 -0.35 -0.27 -0.09 -0.17 -0.11 0.23
SOC 0.61** 0.72*** 0.67** 0.71*** -0.56* -0.58** -0.46* -0.55* 0.67** 0.62** 0.67** -0.55* -0.59** -0.57** 0.18
TN 0.55* 0.36 0.55* 0.50* -0.13 -0.07 -0.23 -0.06 0.55* 0.55* 0.56* -0.06 -0.07 -0.07 0.04
TP 0.02 0.35 0.09 0.18 -0.07 -0.29 0.21 -0.21 0.09 0.02 0.08 -0.21 -0.26 -0.22 0.08
B 0.62** 0.59** 0.63** 0.64** 0.03 0.02 0.02 0.02 0.63** 0.62** 0.63** 0.02 0.03 0.03 -0.03
F 0.39 0.39 0.40 0.41 -0.09 -0.09 -0.07 -0.09 0.40 0.39 0.40 -0.09 -0.07 -0.08 -0.07
AMF 0.50* 0.55* 0.50* 0.54* -0.11 -0.13 -0.03 -0.14 0.50* 0.50* 0.51* -0.14 -0.11 -0.14 -0.07
Act 0.72*** 0.72*** 0.71*** 0.74*** 0.08 0.06 0.09 0.06 0.70*** 0.72*** 0.72*** 0.06 0.08 0.06 -0.09
T-PLFAs 0.64** 0.62** 0.64** 0.66** 0.03 0.02 0.03 0.02 0.64** 0.64** 0.65** 0.02 0.04 0.02 -0.06
MurN GalN GluN T-ASs T-Ass/SOC MurN/SOC GalN/SOC GluN/SOC F-MRC B-MRC T-MRC F-MRC/SOC B-MRC/SOC T-MRC/SOC F-MRC/B-MRC
Correlation
1.0
0.5
0.0
-0.5
-1.0

Fig. 4

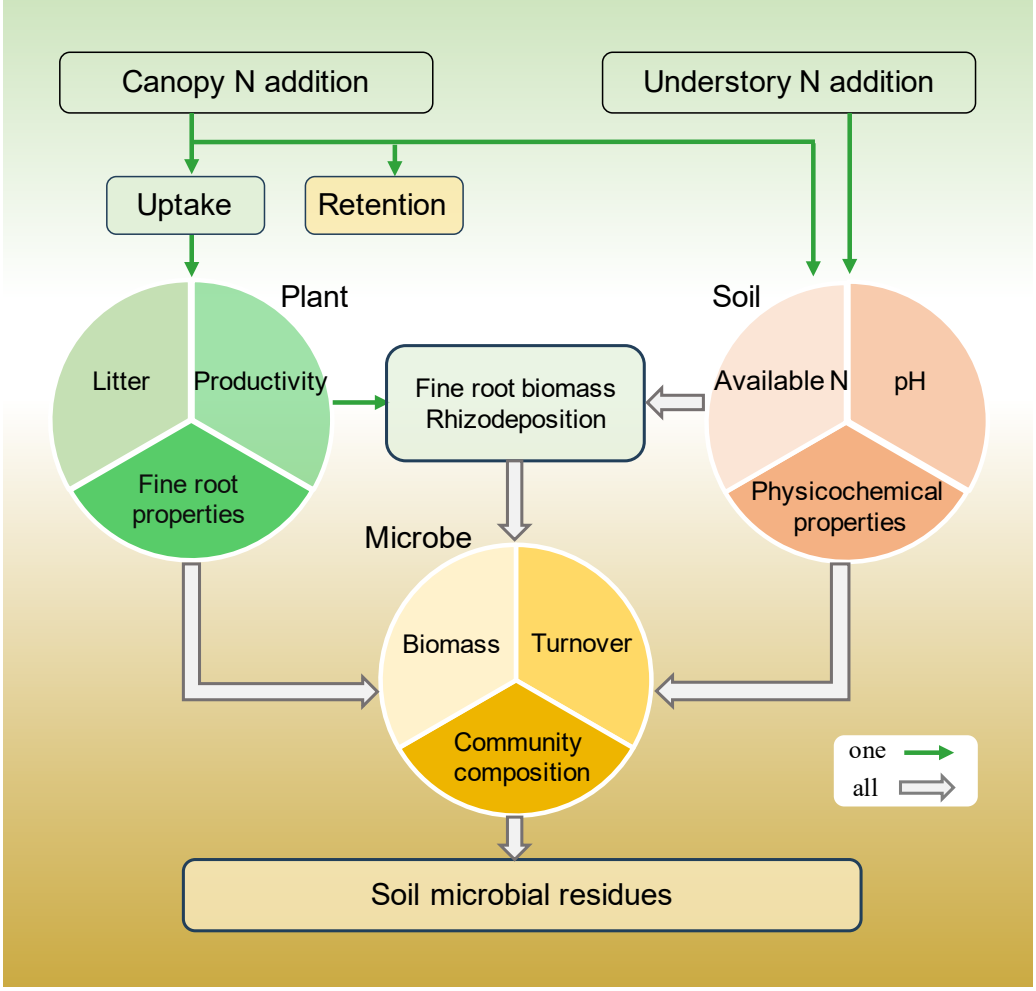
Canopy N addition
Understory N addition
Uptake
Retention
Plant
Soil
Litter
Productivity
Fine root biomass
Rhizodeposition
Available N
pH
Fine root
properties
Physicochemical
properties
Microbe
Biomass
Turnover
Community
composition
one
all
Soil microbial residues

Fig. 5

Table 1 Effects of N addition on soil microbial biomass and community structure by PLFAs methods.

		B	F	AMF	Act	TMB	F: B ratio
0-10 cm	F value	0.34	0.52	0.09	0.50	0.34	1.73
	p value	0.847	0.721	0.985	0.739	0.845	0.197
10-20 cm	F value	0.64	0.26	0.33	1.31	0.71	0.34
	p value	0.642	0.899	0.856	0.312	0.598	0.846

Note: B, F, AMF, Act, TMB, and F:B ratio stand for the biomasses of bacteria, fungi, arbuscular mycorrhizal fungi, actinomyces, and total microbes, and the ratio of fungal to bacterial biomass respectively. F and *p* value is the result of one-way ANOVA. The significant difference was set at the $p = 0.05$ levels.

Table 2 Soil physiochemical properties in all treatments in the 10th years after treatments.

	Treatments	pH	SOC	TN	TP
0-10 cm	CT	4.45a	29.38	1.04b	0.33
	CN25	4.12b	34.88	1.65a	0.27
	CN50	4.18ab	44.09	1.82a	0.34
	UN25	4.10b	37.15	1.37ab	0.30
	UN50	4.11b	39.31	1.69a	0.34
	p value	**0.084**	0.472	**0.096**	0.934
10-20 cm	CT	4.53a	8.96	0.30	0.32
	CN25	4.27b	13.77	0.52	0.23
	CN50	4.37ab	14.88	0.56	0.26
	UN25	4.29b	15.13	0.50	0.31
	UN50	4.31b	13.95	0.46	0.28
	p value	**0.045**	0.386	0.581	0.917

Note: pH, SOC, TN, TP stand for the soil pH value, soil organic carbon, soil total nitrogen, and soil total phosphorus, respectively. Values are means, n = 4. CT, CN25, CN50, UN25, and UN50 stand for the control, canopy N addition at 25 kg N ha^{-1} yr^{-1} and 50 kg N ha^{-1} yr^{-1}, understory N addition at 25 kg N ha^{-1} yr^{-1} and 50 kg N ha^{-1} yr^{-1}, respectively. *P* value is the result of one-way ANOVA in the same soil layer. Different lowercase letters indicate significant differences in the same soil parameter among different treatments in the same soil layer at the $p = 0.05$ levels.